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Research article

Conditioned medium from LS 174T goblet cells treated with oxyresveratrol strengthens tight junctions in Caco-2 cells

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ABSTRACT

Background: Strengthening of intestinal tight junctions provides an effective barrier from the external environment. Goblet cell-derived trefoil factor 3 (TFF3) increases transepithelial resistance by upregulating the expression of tight junction proteins. Oxyresveratrol (OXY) is a hydroxyl-substituted stilbene found in the roots, leaves, stems, and fruit of many plants and known to have various biological activities. In this study, we investigated the strengthening effect of OXY on intestinal tight junctions through stimulation of TFF production in goblet cells.

Methods: We prepared conditioned medium from LS 174T goblet cells treated with OXY (GCO-CM) and investigated the effect of GCO-CM on strengthening tight junctions of Caco-2 cells. The mRNA and protein expression levels of major tight junction components (claudin-1, occludin, and ZO-1) were measured by quantitative real-time PCR and western blotting, respectively. Transepithelial electric resistance (TEER) was measured using an ohm/V meter. Monolayer permeability was evaluated by paracellular transport of fluorescein isothiocyanate-dextran.

Results: OXY showed a strong antioxidant activity. It significantly increased the expression level of TFF3 in LS 174T goblet cells. GCO-CM prepared by treatment with 2.5, 5, and 10 μg/ml OXY did not show cytotoxicity in Caco-2 cells. GCO-CM increased the mRNA and protein expression levels of claudin-1, occludin, and ZO-1. It also significantly increased tight junction integrity and reduced permeability in a dose-dependent manner.

Conclusion: OXY stimulates the expression of TFF3 in goblet cells, which might increase the integrity of the intestinal tight junction barrier.

1. Introduction

In the gastrointestinal tract, tight junctions of epithelial cells form a barrier and regulate absorption of dietary nutrients and defense against intestinal pathogens, allergens, and toxins [1]. Maintaining the integrity of the intestinal barrier is important because increased intestinal permeability exists in several disorders such as obesity, obesity-associated insulin resistance, as well as type 1 and 2 diabetes [2–4]. Intestinal barrier integrity is maintained by intercellular junction complexes such as tight junctions, adherent junctions, and desmosomes [5]. Tight junctions, the most apical complex, consist of claudin-1, occludin, and zonula occludens-1 (ZO-1).

Trefoil factor 3 (TFF3) is a protease-resistant molecule secreted by goblet cells that play a role in mucosal healing by epithelial restitution, mucosal protection, enhancement of the structural integrity of the mucosal barrier, and increasing mucus viscosity [6]. It is also involved in intestinal homeostasis through potentiating epithelial restitution [7,8]. TFF1 and TFF3-overexpressing mice show a protective effect against intestinal damage and ulceration [9,10], whereas mice with deleted TFF1 or TFF3 genes show higher susceptibility to gastrointestinal injury [11,12]. TFFs are expressed under ulcerative conditions of the gastrointestinal tract, such as inflammatory bowel disease characterized by inflammation of the gastrointestinal tract, suggesting that they are important

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molecules involved in the repair of gastrointestinal mucosa [13]. TFFs have a significant role in mucosal repair of the gastrointestinal tract, indicating that TFFs play an essential role in prevention of inflammation and ulceration [14]. TFF3 upregulates claudin-1 and redistributes ZO-1 from the cytoplasm to the intercellular membrane where it binds to occludin, thereby stabilizing intercellular junctions [15].

Reactive oxygen species damage tissues and disrupt cellular functions and structures including tight junctions in the lung epithelial layer [16]. Oxidative stress plays an important role in barrier dysfunction of ventilator-induced lung injury, while superoxide induces dissociation of tight junction complexes [16]. Enhancement of barrier integrity by remodeling tight junctions is mediated by micronutrients, such as zinc, quercetin, butyrate, indole, and berberine [17], and polyphenols such as quercetin, epigallocatechin gallate, resveratrol, and rutin [18]. L-glutamate enhances barrier integrity in response to oxidative stress [19]. All-trans retinoic acid possessing antioxidant properties prevents oxidative stress-induced loss of renal tight junction proteins [20].

Oxyresveratrol (OXY) is a hydroxylated stilbene that possesses an antioxidant activity and anti-inflammatory properties [21]. Therefore, in this study, we investigated whether OXY stimulates production of TFF3 in goblet cells, which increases the expression of occludin, claudin-1, and ZO-1 in intestinal epithelial cells, strengthening intestinal tight junctions. To investigate the strengthening effect of OXY on intestinal tight junctions through TFF3, conditioned medium from LS 174T goblet cells treated with OXY (GCO-CM) was prepared, and expression of tight junction proteins was measured in Caco-2 cell treated with GCO-CM. Human intestinal Caco-2 epithelial cells were used because the Caco-2 cell line is one of the most widely used cell lines to study the assembly of intestinal intercellular tight junctions [22]. In addition, we measured transepithelial electric resistance (TEER) and permeability of fluorescein isothiocyanate (FITC)-dextran, because TEER is indicative of the barrier properties of a monolayer and permeability of FITC-dextran is influenced by intestinal epithelial integrity.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO, USA). Roswell park memorial institute medium (RPMI)-1640, fetal bovine serum (FBS), penicillin/streptomycin (P/S), Eagle's minimum essential medium (MEM), Hank's balanced salt solution (HBSS), nonessential amino acids, and sodium pyruvate were obtained from HyClone (Logan, UT, USA). OXY, FITC-dextran, and dimethyl sulfoxide (DMSO) were purchased from Sigma. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Ameresco (Solon, OH, USA).

2.2. Antioxidant activity of OXY

The DPPH assay was performed using the previously reported method [23] with minor modifications. In 96-well plates, 100 μl of 100 μM DPPH was added to 100 μl of various concentrations of OXY, followed by incubation for 30 min in the dark at room temperature. Then, absorbance was measured at 517 nm using a microplate reader (SpectraMax 340PC 384; Molecular Devices, Sunnyvale, CA, USA). L-ascorbic acid and Trolox were used as positive controls. The DPPH radical scavenging activity was calculated as the 50% inhibitory concentration (IC_{50}).

For the ABTS assay [24], 7 mM ABTS was mixed with 2.45 mM potassium persulfate for 24 h in the dark. ABTS was diluted by mixing with ethanol up to 0.7 ± 0.02 absorbance at 734 nm. Fresh ABTS was prepared for each assay. In 96-well plates, 100 μl ABTS was added to 100 μl of various concentrations of OXY, followed by incubation for 10 min in the dark at room temperature. Then, the absorbance was measured at 734 nm using the microplate reader. L-ascorbic acid and Trolox were used as positive controls. The ABTS radical scavenging activity was calculated as the IC_{50} value.

The ferric reducing ability of plasma (FRAP) assay was performed using the previously reported method with minor modifications [25]. Prior to the experiment, the FRAP solution (ferric TPTZ complex) was prepared by mixing 20 ml acetate buffer (0.3 M, pH 3.6), 2 ml TPTZ solution (10 mM in 40 mM HCl), and 2 ml ferric chloride, followed by incubation for at least 10 min at 37 °C. In 96-well plates, 100 μl of the FRAP solution was added to 100 μl of various concentrations of OXY, followed by incubation for 30 min at 37 °C. Then, the absorbance was measured at 593 nm using the microplate reader. L-ascorbic acid and Trolox were used as positive controls. The reducing activity was calculated as the IC_{50} value.

To measure inhibition of linoleic acid autoxidation, a β-carotene/linoleic acid assay was performed using the previously reported method with some modifications [26]. Briefly, a β-carotene/linoleic acid mixture (300 μl of 1 mg/ml β-carotene dissolved in chloroform, 22 μl linoleic acid, and 200 μl Tween 40) was prepared and diluted with distilled water up to 0.6–0.7 absorbance at 470 nm. To saturate with oxygen, the mixture was shaken vigorously for 30 min in a shaking incubator. In 96-well plates, 180 μl of the β-carotene/linoleic acid mixture and 20 μl of various concentrations of OXY were mixed and the absorbance was measured at 470 nm using the microplate reader at 45 °C every 5 min for 2 h. BHT was used as a positive control. The inhibition activity of oxidation was calculated as the IC_{50} value.

2.3. Cell culture and preparation of conditioned medium from LS 174T goblet cells treated with OXY (GCO-CM)

LS 174T human goblet cell and Caco-2 human colonic epithelial cell lines were purchased from the Korea Cell Line Bank (Seoul, Korea). LS 174T cells were maintained in RPMI-1640 supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Caco-2 cells were maintained in MEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1% nonessential amino acids, and 0.1% sodium pyruvate. The cells were cultured at 37 °C with 5% CO_2. At 80%–90% confluence, the cells were applied to experiments.

Cultured LS 174T cells were gently washed twice with phosphate buffered saline (PBS) and were seeded in fresh serum-free RPMI-1640 medium at a density of 2.5×10^5 cells/ml in 60-mm plates. The cells were cultured overnight at 37 °C with 5% CO_2 and treated with OXY (2.5, 5, or 10 μg/ml). After incubation for 24 h, the supernatants were collected and designated as GCO-CM.

2.4. Cell proliferation induced by OXY and GCO-CM

OXY was dissolved in DMSO. For the MTT assay, LS 174T cells (1.25×10^4/well) were seeded in 96-well plates overnight. Subsequently, the cells were treated with OXY (2.5, 5, or 10 μg/ml). The culture medium was removed and 100 μl of 1:40 diluted MTT in medium was added to each well. The cells were then incubated for 1 h at 37 °C with 5% CO_2. Unreacted dye was removed, and the formazan crystals were solubilized in 100 μl DMSO for 1 h at room temperature. The absorbance was measured at 595 nm using the